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F508del CFTR gene mutation in patients with allergic bronchopulmonary aspergillosis

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ABSTRACT

Objective: The F508del mutation occurs in approximately 3.5% of Caucasian population of Northern Europe. Heterozygotes have increased risk for asthma and reduced pulmonary function. Allergic bronchopulmonary aspergillosis (ABPA) is more common in patients with cystic fibrosis (CF). We aimed to establish the frequency of F508del mutation in adult patients with ABPA. *Methods*: A retrospective matched case-control study of CF genotyped patients with ABPA seen at the National Aspergillosis Centre was undertaken. Key data were collected retrospectively from medical records, including respiratory comorbidities, total IgE, *Aspergillus* IgG and IgE, and immunoglobulins. Cystic fibrosis transmembrane regulator (CFTR) gene mutation analysis included multiplex PCR and sequencing. *Results*: From a cohort of 189 ABPA patients, 156 were screened for common mutations and variants in the CFTR gene. Eighteen were heterozygous for at least one CFTR mutation; 12 (7.7%) were heterozygous for the F508del, notably; 3 were heterozygous for the intron 8 5T variant; and 1 for an intronic variant of uncertain significance, c.3139 + 18C>T. Eight (67%) had asthma, 7 (58%) had CT-defined bronchiectasis, 4 (33%) hypergammaglobulinemia (>16 g/L), 3 (25%) sinusitis and 1 (8%) chronic pulmonary aspergillosis. Eight (67%) had elevated *Aspergillus* IgG antibodies (42–98 mg/L), and 8 (67%) had total IgE above 1,000 KIU/L. Two individuals heterozygous for the F508del mutation and the TG12T5 variant were diagnosed with CF, leading to a *de novo* CF discovery rate of 1.3%. *Conclusions*: In our ABPA patient cohort, the presence of the delta F508 mutation was higher than that seen in general population. Genetic counseling for CFTR genotyping might be appropriate for these patients.

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Introduction

Over 2,000 mutations in the gene encoding the cystic fibrosis transmembrane regulator (CFTR) protein are known [1]. The most common is the deletion of phenylalanine at position 508 (ΔF508), which causes CFTR protein misfolding and retention within the endoplasmic reticulum [2]. This leads to degradation of the protein within the endoplasmic reticulum, which is almost entirely absent at the cell surface. In the homozygous state, subsequent abnormal ion transport leads to absorption of water and thick mucus. The impact of this is particularly seen in the lungs where increased tenacity impairs mucus clearance predisposing infection and lung damage.

F508del CFTR is the most common mutation, occurring in approximately 70% of patients with CF in Northern Europe and North America [3]. In the United Kingdom, about one in 25 individuals is a carrier of a CF mutation [4]. The impact of the F508del mutation in the heterozygous state has not been fully established but has been linked to asthma, chronic obstructive pulmonary disease, and impaired lung function [5].

Allergic bronchopulmonary aspergillosis (ABPA) is a chronic pulmonary disorder secondary to a combined type 1, 3, and 4 hypersensitivity reaction to *Aspergillus* species, typically *A. fumigatus*, leading to an aberrant Th2 response. Disease manifestations differ between individuals but phenotypes include refractory asthma, recurrent pulmonary infiltrates, and bronchiectasis [6]. The environmental and genetic factors contributing to the development of ABPA, and the associated clinical phenotypes, are poorly understood. Previous studies have identified mutations in CFTR as a possible etiologic factor impacting the development of ABPA [7,8].

It is estimated that 6–18% of patients with CF will develop ABPA [9]. In addition, 13% of ABPA patients without CF have been reported to be carriers of a CFTR gene mutation, suggesting a putative role of the CFTR in the pathogenesis of ABPA [7].

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At present, data providing a link between CFTR mutations and pathogenesis of ABPA are limited. Small studies have demonstrated that CFTR gene mutations may result in impaired clearance of *A. fumigatus* spores from the lower respiratory tract [8]. Later work has subsequently demonstrated that CFTR mutations may alter uptake of *A. fumigatus* conidia in murine tracheal cells via an inflammatory mediator cascade resulting in increased expression of *A. fumigatus*-specific $CD4^+$ Th2 cells [10,11]. Clinical evidence in humans is currently absent.

Given that CFTR gene mutations in patients with ABPA are found at a higher frequency than in the general population [8,12] we hypothesized that, heterozygosity for the F508del mutation may predispose the development of ABPA. Furthermore, the rates of ABPA complicating CF are much higher than that seen in individuals without CF. This suggests that predisposition to the development of the disease may be due to the degree of preserved CFTR transporter function. This study explored the hypothesis that amongst patients with ABPA, in the absence of CF, the F508del carrier state occurs more frequently than in the general population.

Methods

A 1:3 retrospective case-control study matching age and gender (age range $\pm$ 2 years) was undertaken. The initial cohort consisted of patients with ABPA referred to the National Aspergillosis Centre (NAC), University Hospital of South Manchester, within the study period from 2008 to 2014. The diagnosis of ABPA was established by cross-referencing information provided in the initial referral letters, ensuring radiological and serological compatibility with ABPA diagnosis. The resultant cohort was further filtered to include only those patients where CF genotyping was available. To assess for allelic frequency of the F508del mutation, and to generate population homogeneity, only heterozygotes for the F508del gene mutation were included in the final cohort. If genotyping analysis revealed mutations consistent with a possible diagnosis of CF then sweat testing was undertaken.

Inclusion criteria

Inclusion criteria included history of asthma or bronchiectasis, evidence of ABPA, and possible co-existence of chronic pulmonary aspergillosis (CPA) or *Aspergillus* sinusitis. Subjects were above 18 years of age. Patients were screened for CF via genetic testing and a sweat test when indicated, as mentioned above. For reasons of accuracy and consistency, cases were diagnosed as ABPA based on history, radiographic pulmonary opacities consistent with ABPA, elevated total IgE level (>1,000 IU/mL), elevated specific IgE levels against *A. fumigatus*, and presence of precipitating or IgG antibodies against *A. fumigatus.* Consistent with the proposed new diagnostic criteria for ABPA, patients with a total IgE of <1,000 IU/mL were classified as having ABPA, providing all other diagnostic criteria were met [6]. This allowed inclusion of patients with a clear disease phenotype where therapy may have lowered total IgE.

Exclusion criteria

Patients with severe asthma with fungal sensitivity, *Aspergillus* bronchitis, or allergic bronchopulmonary mycosis, where disease phenotype is driven by fungi other than *Aspergillus* spp., were not enrolled.

Data collection

The following retrospective data were collected from the medical records of each individual within the final cohort. Data were divided into demographic details (age and gender); diagnosis (primary diagnosis and associated co-morbidities for example bronchiectasis, asthma, and sinusitis); immunological and serological biomarkers; mannose binding lectin (MBL), total IgE, serum immunoglobulins, *Aspergillus* IgG, *Aspergillus* IgE, C-reactive protein (CRP), *Haemophilus influenza*, and Pneumococcal serology; measures of pulmonary function and associated limitation via the Medical Research Council (MRC) dyspnea score, as well as forced expiratory volume in the first second (FEV_1). The result of CF genotyping was evaluated, looking specifically for the presence of the delta F508 mutation.

Serology and immunology

Total IgE, *Aspergillus* IgE, and *Aspergillus* IgG were assayed using the semi-automated Phadia ImmunoCap® method (ThermoFisher). The cut-off levels for the *Aspergillus* IgG assay have been determined by comparison to normal sera, from healthy donors (<40 mg/L) and have been consistent for over 25 years. *Aspergillus* IgE is regarded as abnormal if any is detectable, so it is set at the limit of detection of the assay (>0.35/0.4 kAU/L). MBL was measured using an enzyme-linked immunosorbent assay (ELISA) (MBL Oligomer ELISA kit; Bio-Porto Diagnostics A/S, Gentofte, Denmark) (range 50–4,000 ug/L). Immunoglobulins and electrophoresis were measured using quantitative nephelometry and capillary zone electrophoresis and immunofixation. All tests were assayed by the Immunology Department, Manchester Royal Infirmary. Pneumococcal and *Haemophilus* antibody levels were measured by the Public Health England Vaccine Evaluation Unit (Manchester Royal Infirmary).

Cystic fibrosis molecular diagnostics

Genetic testing was performed at the Genomic Diagnostics Laboratory at the Manchester Centre for Genomic Medicine. A multiplex PCR for detection of wild-type and mutated alleles in the CFTR gene was used. In the presence of extensive bronchiectasis and a single mutation, mutations were confirmed using Sanger sequence analysis. The Devyser Core kit is designed to detect mutations common to the White British population and the UK kit detects mutations common in the Pakistani population within the United Kingdom [13].

The Devyser CFTR-Core kit is designed to genotype the normal and mutant alleles at 33 loci of the CFTR gene using purified human genomic DNA. Genotype coverage includes a panel of 36 mutations to support genetic diversity. The assay also detects polythymidine variants (5T/7T/9T) within intron 8 (IVS8) of the CFTR gene. In case of a 5T allele, the TG repeat number upstream of the poly-T region can also be determined. The Devyser CFTR Core kit is based on multiplex allele-specific PCR amplification for detection of normal non-mutated (wild-type), and mutated alleles in the CFTR gene. Allele-specific PCR amplification generates fluorescently labeled fragments that are analyzed by capillary electrophoresis on a Genetic Analyzer instrument.

The Devyser CFTR UK kit is designed to genotype the normal and mutant alleles at 17 loci of the CFTR gene using purified human genomic DNA. Genotype coverage includes a panel of 17 mutations to support the detection of mutations specifically found in the UK population, including common Celtic, Chinese, and Pakistani mutations. The Devyser CFTR UK kit also includes the analysis of cross-mix ID markers for sample identity confirmation compatible with all Devyser CFTR products.

Statistical analysis

Patients with CFTR mutations and ABPA were matched for gender and age (within $\pm$ 2 years). Demographic variables were tabulated as median and range, where appropriate. Continuous and categorical variables were analyzed using Mann–Whitney *U* and Fisher's exact tests, respectively. A *P*-value of <0.05 was considered to be statistically significant.

Results

Demographics

From a total cohort of 189 ABPA patients, 156 were genetically screened for CF. Eighteen (11.5%) were found to possess CFTR mutations; twelve patients (7.7%) demonstrated the F508del CFTR mutation; and six other CFTR-related mutations. Regarding ethnic background, all 12 patients with the F508del CFTR mutation were Caucasians.

Clinical manifestations

Of the 12 patients with the F508del CFTR mutation, 8 (67%) had asthma, 7 (58%) had CT-defined bronchiectasis, 3 (25%) rhinosinusitis, and 1 (8%) CPA. There were no significant differences in clinical manifestations between index patients and control patients (Table 1).

Laboratory features

Five patients (42%) had MBL deficiency (<1 mg/L), eight (67%) had elevated *Aspergillus* IgG antibodies (42–98 mg/L), and 8 (67%) had total IgE antibodies above 1,000 IU/L. All twelve individuals with the delta-508 CFTR mutation had elevated anti-*Aspergillus* IgE (0.8–80 kAU/L). Of note, none displayed hypogammaglobulinemia, and 4 (33%) had hypergammaglobulinemia (>16 g/L). There were no significant differences in maximum CRP values between the two populations (Table 1).

Airway obstruction and functional disability

There were no significant differences between study groups in baseline, current, and lowest FEV_1 and MRC dyspnea score (Table 1).

Genotyping

CF genetic results in patients with ABPA are displayed in detail in Table 2. Eighteen of the patients genotyped demonstrated mutations within CFTR. Twelve individuals were heterozygous for the F508del mutation. In the remaining five cases, other allelic variants, predominantly intron 8, were present. The remaining six ABPA patients had other CFTR mutations. Overall, the frequency of CFTR mutations, including F508del, was 11.5% in the sampled population.

Two patients (heterozygous 508 delta mutation and TG12T5 intron 8 variant) were newly diagnosed with CF disease; one individual presented at NAC with ABPA, bronchiectasis, recurrent rhinosinusitis, and gastroesophageal reflux disease, and then, at the age of 59 years, genotyping found CF corresponding to a mild phenotype (positive sweat test, prior history of pancreatitis. and a history of asthma) and the other patient, with a history of ABPA without asthma, was referred to the CF unit when he was 72 years of age (sweat test failed). Overall, the

Table 1. Comparison of allergic bronchopulmonary aspergillosis (ABPA) patients with F508del CFTR gene mutation vs age and gender-matched control ABPA patients without F508del CFTR gene mutation.

Variable	F508del CFTR + ABPA ($N = 12$) *n* (%)	ABPA controls ($N = 36$) *n* (%)	*P*-value[a]
Asthma	8 (67)	26 (72)	
Age in years (median, range)	64 (48–76)	64 (47–76)	
Gender (male)	8 (67)	24 (67)	
Bronchiectasis	7 (58)	20 (56)	
CPA[b]	1 (8)	1 (3)	
Rhinosinusitis	3 (25)	0 (0)	0.013
IgE (1,000–1,499 KIU/L)[c,d]	3 (25)	4 (11)	
IgE (1,500–1,999 KIU/L)[c,d]	2 (17)	7 (19)	
IgE (≥2,000 KIU/L)[c,d]	4 (33)	16 (44)	
A. fumigatus IgE (0.4–3.99 kAU/L)[e]	1 (8)	6 (17)	
A. fumigatus IgE (4.0–39.99 kAU/L)[e]	8 (67)	15 (42)	
A. fumigatus IgE (≥40 kAU/L)[e]	3 (25)	15 (42)	
MRC dyspnea score (median, range)[f]	2.0 (1–5)	1.96 (1–5)	
Current MRC dyspnea score (median, range)	2.0 (1–5)	2.59 (1–5)	
Max MRC dyspnea score (median, range)	3.0 (1–5)	3.24 (1–5)	
Baseline FEV_1 (median, range)[g]	2.14 (0.47–3.84)	2.05 (0.44–4.49)	
Current FEV1 (median, range)	2.38 (1.8–2.73)	1.97 (0.55–3.29)	
Lowest FEV1 (median, range)	1.78 (1.1–2.73)	1.83 (0.44–3.29)	
CRP in mg/L (mean, range)[h]			
Baseline CRP (mean, range)	15 (4–98)	21.4 (<5–72)	
Maximum CRP (mean, range)	25 (4–98)	74.6 (6–303)	
Non-protective *H. influenzae* antibody titers[i]	3 (25)	17 (47)	
Non-protective pneumococcal antibody titers[j]	2 (17)	7 (19)	
Hypergammaglobulinemia (IgG >16 g/L)	4 (33)	0 (0)	0.025
Hypogammaglobulinemia (IgG <7 g/L)	0 (0)	6 (17)	
MBL deficiency (<1.0 mg/L)[k,l]	5 (42)	13 (36)	

[a] *P*-values not shown were not significant.
[b] CPA: chronic pulmonary aspergillosis.
[c] Total IgE (ABPA cases): mean 1,192 (130–7,500); 3 cases had total IgE <1,000 KIU/L.
[d] Nine controls had total IgE <1,000 KIU/L.
[e] *Aspergillus fumigatus* IgE (ABPA cases): mean 32.4 (0.8–80).
[f] MRC dyspnea score: Medical Research Council dyspnea score.
[g] FEV_1: forced expiratory volume in the first second.
[h] CRP: C-reactive protein.
[i] Adequate influenza serology: *Hemophilus influenza* antibodies >0.15 ug/mL.
[j] Adequate pneumococcal serology: ≥7 *Pneumococcus* serotypes >0.35 ug/mL.
[k] MBL deficiency: mannose binding lectin deficiency.
[l] Mean MBL (ABPA cases): mean 0.98 (0.05–2.62).

de novo discovery rate of undiagnosed CF complicating ABPA in this population was 1.3% (2:156).

Discussion

Our study shows that hypergammaglobulinemia and rhinosinusitis are significant phenotypic variables in patients with the F508del mutation presenting with ABPA. Whilst the presence of ABPA in the context of CF is well described, the implications of carrying a CFTR mutation are not well established for other respiratory pathologies.

Table 2. Cystic fibrosis genetic results in patients with allergic bronchopulmonary aspergillosis.

Genotype	*N*
F508del/—	7
F508del/TG12T5 intron 8 variant*	3
F508del/TG11T5 intron 8 variant	1
F508del/c.3139 + 18C>T	1
TG12T5 intron 8 + Gly551Asp/—	1
c.1652G>A p.(Gly551Asp)/—	2
c.1624G>T p.(Gly542Ter) /—	1
c.489 + 1G>T/—	1
Arg(117)His in cis with intron 8 Poly7T/—	1

*Two patients among three, who were heterozygous for F508del and TG12T5 intron 8 variant, were diagnosed with CF.

An increased frequency of the CFTR mutations has previously been demonstrated in the ABPA population [7,8,12,14]. Lebecque and colleagues [14] studied 18 adult patients with ABPA and found that the CFTR mutation carrier frequency was 67%. However, the functional consequences are as yet unclear. We found that the overall frequency of mutations within CFTR in the NAC ABPA population was 11.5% with the frequency of the F508del mutation being 7.7%, exceeding that of the general population. Our findings are also consistent with those of Agarwal et al. [15], where extensive review for CFTR mutations in the ABPA population found that the odds ratio of CFTR mutation was higher in ABPA compared to control group (OR 10.39; 95% CI, 4.35–24.79).

Our data analysis suggests that hypergammaglobulinemia ($P = 0.025$) and rhinosinusitis ($P = 0.013$) are more

prevalent in ABPA individuals with the F508del mutation compared to those without this mutation. This might indicate that these characteristics may be part of a specific disease phenotype of the ABPA patient population. Interestingly, despite the role of CFTR mutation in bronchiectasis associated with CF, an increase in bronchiectasis was not seen in the affected heterozygotes. Functional disability conferred by breathlessness was also similar between the two groups.

Serum MBL concentrations have been associated with aspergillosis. MBL deficiency correlates with predisposition, clinical manifestations, and progression of invasive pulmonary aspergillosis [16]. Several studies have found varied degrees of association between MBL genotypes and functional protein levels with chronic and allergic aspergillosis [17,18], and deficiency is associated with more breathlessness in ABPA [17]. In our population, we did not detect any significant difference of MBL deficiency when comparing ABPA patients with F508del CFTR gene mutation to ABPA patients without it.

The demonstration of higher levels of documented rhinosinusitis in patients with ABPA and F508del mutations compared to controls ($P = 0.013$) may represent a possible novel association between CFTR mutation and sinus disease in the heterozygous state. Allergic *Aspergillus* rhinosinusitis commonly complicates ABPA and it is interesting that rates appear higher in individuals with the delta-F508 mutation [19]. If it is proven that heterozygosity for CFTR F508del mutation impacts on mucus composition and subsequent mucus clearance, the increased frequency of rhinosinusitis in our patients may be a reflection of the impact of impaired clearance of fungal elements from the upper airways. Continued exposure to *Aspergillus* antigen via persistent hyphae in the paranasal sinuses may then increase the frequency of clinically overt sinusitis in ABPA.

Our study design precluded identification of individuals with chronic sinus problems and those with documented one-off episodes. It was also impossible to establish whether documented episodes were bacterial, fungal, or allergic in origin, further complicating evaluation of the potential role of CFTR. Establishing rhinosinusitis as a phenotypic feature of individuals with ABPA and F508del mutation, therefore, requires further prospective evaluation.

The identification of increased frequency of hypergammaglobulinemia in this population also raises significant questions. Given that a polyclonal gammaglobulin increase has been associated with a multitude of conditions, including chronic infection and inflammation, it may be that the apparent differences merely represent chronic suppurative infection in the context of bronchiectasis and airways inflammation, co-morbid conditions, or the time of sampling [20]. Nonetheless, it is possible that reduced CFTR function generates abnormal mucus secretions increasing the risk of endobronchial colonization with agents like *Pseudomonas aeruginosa*. This might increase predisposition to recurrent infections and a chronic proinflammatory response leading to hypergammaglobulinemia. The microbiological profiles of this cohort were not systematically studied and this may be a valuable area for future study.

Within the CF population, hypergammaglobulinemia is indicative of more advanced lung disease. Jones and colleagues [21] found that CF patients who develop hypergammaglobulinemia have circulating immune complexes and relatively severe associated disease, further underscoring the prognostic importance of hypergammaglobulinemia as a marker for progressive CF disease. This might suggest that persistent hypergammaglobulinemia in ABPA, irrespective of CFTR mutation status, may be a sign of a more advanced disease. The significance of hypergammaglobulinemia in the F508del ABPA, and wider ABPA population remains an area for further study.

The absence of increased bronchiectasis in our patients heterozygous for the F508del mutation is interesting. Girodon et al studied 32 patients with severe bronchiectasis without ABPA, and found 13 CFTR gene mutations in 16 different alleles, suggesting a putative role for CFTR in the pathogenesis of bronchiectasis [22]. Given that the prevalence of bronchiectasis in our ABPA population was not significantly different between the two groups, the role of CFTR in the context of *Aspergillus* sensitization and ABPA remains unclear. It is possible that CFTR mutations may play a role in the development of bronchiectasis but these mutations are heavily modulated by epigenetic and environmental influences. The fact that not all patients with ABPA have bronchiectasis further complicates this issue.

The impact of identification of CFTR mutations in a small number of individuals with ABPA raises the possibility of the use of CFTR channel potentiators, such as lumacaftor and ivacaftor in this subpopulation. Perhaps reduced functional CFTR expression would be increased by drug administration leading to improved clearance of airway pathogens (bacterial and fungal), thus decreasing inflammation. Ultimately, this would lead to long-term improvement in respiratory reserve and preservation of lung architecture with reduction in bronchiectasis. Clinical trials, evaluating lumacaftor/ivacaftor combination therapy in patients with CF who have a delta-F508del CFTR mutation, have shown variable impact on mean sweat chloride concentration [23,24]. However, these drugs have not been tested outside of the setting of CF, though they have proved effective in patients with CF and ABPA [25,26]. Further translational work to assess

the impact of F508del and other CFTR mutations in the heterozygotes would be required prior to human studies.

Among the limitations of this study is its retrospective nature. The possibility of sample bias in the studied population has been discussed earlier. Nonetheless, to our knowledge, this is the largest study in adult ABPA population assessing the frequency of the F508del mutation. Our data provide evidence that there is a potential link between the presence of CFTR mutations and a specific disease phenotype in individuals with ABPA. A prospective study of all individuals referred with ABPA over prolonged periods of time is required, perhaps combined with a more comprehensive genetic analysis. This study was not controlled for previous or current use of antifungal therapy, which may have significantly altered disease phenotype, as well as serological and immunological markers. Additionally, some patients had extended screening of the whole gene, whereas others did not. So, there is a small chance that these patients might have a second unidentified mutation. However, our data provide additional insight into the pathogenesis and possible phenotypic impact of CFTR mutations in a well-defined and closely followed cohort of ABPA patients at the NAC.

Conclusions/key findings

Confirmation of a higher rate of carriage of CFTR, including F508del mutation, raises the possibility of genetic counseling and screening for affected individuals. We found two patients with newly diagnosed CF (1.3%). Clinicians should remain alert to the possibility of undiagnosed CF in individuals with ABPA with or without severe bronchiectasis. Research into the impact of CFTR mutations on patients with ABPA without CF may provide avenues for further treatment, which are currently sorely needed.

Declaration of interest

All authors declare that they do not have a commercial or other association that might pose a conflict of interest for this specific work.

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